

Amendments to the Specification:

Please replace the paragraph beginning on page 9, line 9 with the following paragraph:

The level of protein homology can be determined with the computer program "BLAST 2 SEQUENCES" by selecting sub-program: "BLASTP", that can be found at www.ncbi.nlm.nih.gov/blast/bl2seq/bl2.html, the United States' National Institutes of Health's National Library of Medicine's National Center for Biotechnology Information world wide web site. A reference for this program is Tatiana A. Tatusova, Thomas L. Madden FEMS Microbiol. Letters 174: 247-250 (1999). Matrix used: "blosum62". Parameters used are the default parameters: Open gap: 11. Extension gap: 1. Gap x_dropoff: 50.

Please replace the paragraph beginning on page 16, line 6 with the following paragraph:

The level of nucleotide homology can be determined with the computer program "BLAST 2 SEQUENCES" by selecting sub-program: "BLASTN" that can be found at www.ncbi.nlm.nih.gov/blast/bl2seq/bl2.html, the United States' National Institutes of Health's National Library of Medicine's National Center for Biotechnology Information world wide web site. A reference for this program is Tatiana A. Tatusova, Thomas L. Madden FEMS Microbiol. Letters 174: 247-250 (1999). Parameters used are the default parameters: Reward for a match: +1. Penalty for a mismatch: -2. Open gap: 5. Extension gap: 2. Gap x_dropoff: 50.

Please replace the paragraph beginning on page 25, lines 4-28 with the following paragraph:

Expression and purification of GST-ORF1 and GST-ORF2 recombinant proteins in *E. coli*.

In order to express the products encoded by ORF1 and ORF2 from the *BCVIR* cDNA as recombinant proteins, and to produce specific antisera against them, the two ORFs were subcloned in the *EcoRI/XhoI* cut dephosphorylated pGEX-4T3 vector (Amersham Pharmacia Biotech) in frame with the glutathione-S-transferase (GST). In order to produce Bcvir15 protein encoded by ORF1 (aa M¹-I¹⁴¹, Fig.2), the *EcoRI/XhoI* fragment containing the 1041 bp cDNA sequence (missing the 5' end of *BCVIR* cDNA) was excised from the pBK-CMV recombinant plasmid and subcloned into the vector. In order to obtain an antiserum specifically directed against the amino acid sequence deduced from the ORF2 sequence (aa sequence N¹⁵⁴-C²⁸⁵, Fig.3), *i.e.* excluding the 52 bp overlapping region between the two ORF (nucleotide position 448-500 in Fig.1), an *EcoRI* restriction site was created in the ORF2 sequence by a PCR experiment. Thus, the 1041 bp *BCVIR* cDNA sequence carried by the pBK-CMV plasmid was used as a template DNA for the PCR experiment with the sense primer P_{Eco} (5'-ATGAGGAATTCGAACCGACTA-3' (SEQ ID NO.: 10); located at nucleotide position 529-549 on the complete *BCVIR* cDNA sequence, Fig.1 and Fig.2) and the antisense T7 universal primer derived from the vector sequence (located 75 bp downstream of the *XhoI* cloning site). The amplified fragment was cloned in pGEM[®]-T vector using the pGEM[®]-T vector System II kit under the recommendations of Promega. An *EcoRI/XhoI* fragment containing an around 600 bp DNA from ORF2 was released from the recombinant plasmid and then subcloned into the vector. For both clonings, transformation with the ligation mixtures were performed into the *E. coli* BL21 cells and positive clones were selected using PCR with the specific primers from the *BCVIR* cDNA sequence.

Please replace the paragraph beginning on page 27, line 32 and extending to page 28, line 25 with the following paragraph:

In Vitro translation of the BCVIR cDNA sequence. The ability of the *BCVIR* cDNA to direct frame shifting was examined in an *in vitro*-translation system. *In vitro*-translation products were synthesised using the TNT[®] Quick Coupled Transcription/Translation System (Promega) with a PCR DNA fragment containing the entire *BCVIR* cDNA sequence, a Kozacks sequence and a T7 promoting sequence at its 5' end for the *in vitro* translation. In order to amplify such a DNA fragment, the PCR was performed on the pBK-CMV plasmid carrying the incomplete 1041 bp *BCVIR* cDNA as DNA template using the two following primers: a 90 mer sense oligonucleotide (primer P0 : 5'-

GGATCCTAATACGACTCACTATAGGGAGACCACCATGGAGTCGACATCAACAG
ACCAACTTTGTTGCCGAGAACCGTCCCACCTTTGG-3' (SEQ. ID. NO.: 16)), containing a Kozacks and a T7 promoting sequences but also the missing 5' end of the entire *BCVIR* cDNA sequence containing the starting ATG codon of the ORF1 (bolded in the primer sequence; nucleotide position 75-130 on the complete cDNA sequence; see Fig.1 and Fig.2), and the antisense primer P15.2 from the *BCVIR* cDNA sequence. The PCR was performed using 100 ng of the circular recombinant pBK-CMV plasmid and the following conditions of amplification were used: 1 cycle of 3 min. at 94°C; 5 cycles of 1 min. at 94°C, 1 min. at 37°C and 1 min. at 72°C; 25 cycles of 1 min. at 94°C, 1 min. at 50°C and 1 min. at 72°C. The PCR products were gel extracted twice to ensure their purity and resuspended in nuclease free water at a concentration of 100 ng/μl. Then, they were added into a reticulocyte lysate to be translated using the TNT[®] Quick Coupled Transcription/Translation System as described by the manufacturer (Promega). As control, the same experiment was performed by omitting the PCR fragment in the reaction. The total radiolabeled translated products (5 μl of the reaction) and immunoprecipitated

products (from 20 µl of the reaction) with the anti-GST-ORF1 or the anti-GST-ORF2 or the anti-GST antisera and the pre-immune rabbit serum were separated on a 15% SDS-PAGE.

Please replace the paragraph beginning on page 37, line 2 with the following paragraph:

Figure 1: Schematic representation of the *BCVIR* cDNA

A: Schematic representation of entire *BCVIR* cDNA with predicted ORFs represented by boxes and sequence derived primers represented with arrows.

B: Representation of the predicted overlapping sequence (SEQ ID NO.: 11) and its deduced amino acid sequences ORF2 (SEQ ID NO.: 17) and ORF1 (amino acids 123-141 of SEQ ID NO.: 2).

Please replace the paragraph beginning on page 37, line 8 with the following paragraph:

Figure 2: Nucleic sequence of the complete *BCVIR* cDNA (SEQ ID NO.: 1) and deduced amino acid sequence (SEQ ID NO.: 2) of the Bcvir15.

The common amino acid sequence between Bcvir15 and the putative Bcvir32 is represented in regular upper case. A GRAM-positive anchoring hexapeptide is overlined. The overlapping sequence between the two predicted ORF of *BCVIR* cDNA are indicated in bold italic. Cloning restriction sites are in **bold case**.

Please replace the paragraph beginning on page 37, line 15 with the following paragraph:

Figure 3: Nucleic sequence of the complete *BCVIR* cDNA (SEQ ID NO.: 3) and deduced

amino acid sequences of the putative Bcvir32 protein (SEQ ID NO.: 4).

The overlapping region between ORF1 and ORF2 of the *BCVIR* cDNA is indicated in ***bold italic***.
Cloning restriction sites are in **bold case**.

Please replace the paragraph beginning on page 38, line 21 with the following paragraph:

Figure 8: Immunoprecipitation of [³⁵S] methionine labelled *Babesia canis* isolate A antigens
Radiolabelled Total antigens (A) or Exoantigens (B) were immunoprecipitated respectively by rabbit pre-immune sera (1), anti-GST rabbit polyclonal serum (2), anti-GST-ORF1 rabbit polyclonal serum (3) and anti-GST-ORF2 rabbit polyclonal serum. ~~Arrows and asterisk~~ Asterisk and arrows indicate respectively the location of the Bcvir15 and the putative Bcvir32.